"INDIRECT" ISOSEROLOGIC INCOMPATIBILITY IN EXPERIMENTAL TRANSFUSION AND ITS CLINICAL SIGNIFICANCE

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Many aspects of the problem of group-dependent isoserologic properties of the blood in dogs [1, 13, 14], animals nowadays widely used in experimental transfusion research, still remain unexplained. In blood transfusion and artificial circulation experiments on dogs a simplified technique of screening by cross-matching is used: In a previously selected pair of animals (according to body weight, age, and other parameters) a cross-matching test for red cell agglutination is carried out, and if the test is negative, the pair of animals is considered to be compatible and suitable for exchange blood transfusion. At major research centers, where experiments are carried out on nursery-reared animals with considerable genetic homogeneity, such a system is perfectly adequate. However, in laboratories which obtain their animals from other sources (rounded up strays), this method of screening does not guarantee complete isoserologic compatibility of dogs of different breed, more especially of mongrel dogs. This state of affairs has led some workers to the incorrect conclusion that there exists a massive blood transfusion syndrome [4, 10-12], which the writers have already discussed previously [6-8], on the basis of their own experimental and clinical investigations. It was shown that if, in experiments on dogs, truly isoserologically compatible animals are selected, no features which can be ascribed to such a syndrome are observed. The same also was true of the clinical use of an artificial circulation, which can be regarded as a variant of massive blood transfusion. However, it is quite clear that this is not the end of the problem, for doubts about the advisability of clinical use of the artificial circulation method, the basis of modern heart surgery, still remain.

The main aim of this investigation was an experimental analysis of the view that any blood transfusion on dogs in a volume of 45-50 ml/kg inevitably leads to the development of toxemia, the principal sign of the massive blood transfusion syndrome [3], in these animals.

EXPERIMENTAL METHODS

Experiments were carried out on mongrel dogs. Animals weighing not more than 7-8 kg were selected as recipients, and dogs weighing 18-20 kg were used as donors. This facilitated the preparation of a sufficient volume of blood, and all transfusions given were "massive" (not less than 45 ml/kg body weight). Blood was prepared in heparin (40 mg/liter) on the day of the experiment. The animals were premedicated with trimeperidine in a dose of up to 10 mg/kg body weight. For anesthesia the donors were given an intravenous injection of hexobarbital (up to 30 mg/kg, in 2.5% solution), and the recipients were given neuroleptanalgesia with fentanyl and roperidol (in the usual doses). Intra-arterial injection from a Bobrov apparatus was used for blood transfusion, and a cannula was introduced into one femoral artery for this purpose. Immediately before the injection, the animal was bled through the cannula in a volume of 30-35 ml/kg. Some special comments must be made on the technique of choice of isoserologically compatible pairs of animals. The day before the experiment a group of seven or eight animals was first selected in the dog house (on the basis of weight and other parameters), and 5 ml blood was taken from a vein of each animal into tubes. The blood was centrifuged to obtain packed red cells and serum from each dog. Compatibility testing was then carried out between all animals of the group, by mixing red cells from each dog with sera from all the rest of the group separately on slides. The results of the tests (presence or absence of agglutination) were tabulated. On the basis of

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these results the experiments were divided into three goups. In group 1 (four experiments) each pair of animals (recipient and donor) were definitely incompatible, i.e., the donor's red cells were agglutinated by the recipient's serum. In group 2 (three experiments) the pairs were compatible according to our criteria: The donor's red cells were not agglutinated by the recipient's serum, not only in each pair, but also in all the other tests. Finally, in group 3 (21 experiments) the donor's erythrocytes in each pair were not agglutinated by the recipient's serum, but they were agglutinated in tests with serum from two or three other dogs in the same group. These pairs of animals were conventionally described as indirectly incompatible. The experiments of group 3 were divided in turn into two subgroups: In the first subgroup blood was transfused under normothermic conditions, in the second subgroup (six experiments) under hypothermic protection of the recipient. The animals were cooled by the immersion method, i.e., through the skin by application of ice bags. The temperature in the esophagus was lowered to 20-22°C. After the end of transfusion, the animals were reheated by means of hot water bottles.

EXPERIMENTAL RESULTS

In the experiments of group 1 the animals died actually during blood transfusion. The principal pathophysiological manifestation of isoserologic incompatibility were severe disturbances of the hemodynamics and cardiac activity, arterial hypotension and the development of ventricular fibrillation, subsequently changing to asystole. No resuscitation measures were able to restore cardiac activity.

In the experiments with complete compatibility (group 2) the blood transfusion was uneventful. The animals survived and were completely viable. After a long period of observation no abnormality was found at autopsy.

In the experiments of group 3 with normothermic transfusion a tendency toward arterial hypotension also was observed, together with extrasystoles, ventricular fibrillation, and other disorders. By intracardiac injection of adrenaline and calcium chloride, direct cardiac massage, and electrical defibrillation, cardiac contractions were restored in these experiments. Soon, however, they were disturbed again, with the addition of a bleeding tendency, despite adequate neutralization of heparin by protamine sulfate. Of the 15 experiments in this subgroup, long-term survival of the animals did not occur in any single case: Death ensued either during the first few hours or the first day.

In the experiments of group 3 with hypothermic protection of the recipients the course was different. All the experimental animals tolerated the blood transfusion, and four of them remained under observation for 3 weeks, during which time their behavior was indistinguishable from that of intact animals. These dogs were autopsied after this period and no abnormality was found in their organs.

We know that immunologic reactions do not correspond either in character or intensity of formation to the "all or nothing" formula [5]. This situation was further confirmed by the present experiments in the field of transfusion reactions. By the method of isoserologic screening of animals which we used in this investigation, it is possible to reproduce deliberately either more or less serious degrees of isoserologic incompatibility in animals. The less serious forms are obtained by the use of "indirectly" incompatible animals. A similar view on the existence of gradations of incompatibility is held by Fedorov and Movshev [12], who consider that different degrees of isoserologic compatibility must be distinguished. However, it is more correct to speak of gradations of incompatibility, for treatment in clinical practice under these circumstances may be different, as our experience of the use of hypothermic protection in animals indicates.

This investigation also has cleared up some uncertainties with regard to the massive blood transfusion syndrome. Everything ascribable to this syndrome is a manifestation of a certain degree of isoserologic incompatibility, more especially because, in animals surviving blood transfusion because of hypothermic protection, despite the presence of "indirect" incompatibility, on the day after the experiment the presence of toxemia was discovered [3]. The syndrome was thus entirely due, not to the size of the blood transfusion, but simply to incomplete isoserologic compatibility of recipient and donor. It is evident that with improvements in techniques for testing isoserologic compatibility in clinical transfusion practice, to which all efforts must be directed, the problem of the "syndrome" will be solved. At the same time, of course, the above remarks must not be taken to imply that the size of transfusions is unimportant. On the contrary, with an increase in the

volume of blood transfused and, consequently, with an increase in the number of donors whose blood is used for this purpose, there is a greater chance of encountering a recipient with antigen systems of the M, N, and S type (outside the ABO and rhesus systems), which are probably involved in blood transfusion reactions with a rather vague clinical picture.

It can be postulated that traces of mild forms of isoserologic incompatibility, which because of the severity of the operation itself may remain undiagnosed, are more probable in cardiac surgical practice with the use of an artificial cirulation and of large volumes of donated blood. However, these reactions may contribute to the development of acute heart failure in patients in the early postoperative period [2].

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